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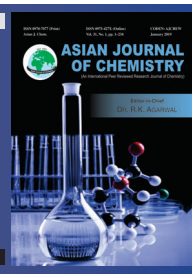

Isolation and Structural Characterization of Degradation Products of Aceclofenac by HPLC, HRMS and 2D NMR

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The stability of aceclofenac under stress conditions was assessed to identify the degradation products. So, it was subjected to stress conditions like acid, base and oxidation, according to ICH guideline Q1A (R2). One degradation product formed when the drug was subjected to acid stress. Three degradation products were formed during the basic stress condition. The drug substance was found to be stable to oxidative stress. The degradants formed during the stress were separated on a C-18 column using gradient preparative HPLC elution. The only product (**DP-2**) formed during the acid stress and this one is same as of one of the three degradation products (**DP-1**, **DP-2**, **DP-3**) were formed during base stress. 1D and 2D NMR spectra and mass spectral analysis supported the proposed structures for the products. The products **DP-2** and **DP-3** have been reported earlier but this is the first report of product **DP-1** as a degradation product of aceclofenac.

Keywords: Aceclofenac, Degradation products.

INTRODUCTION

Aceclofenac belongs to a class of non-steroidal antiinflammatory drugs (NSAIDs). It is prescribed for the relief of pain and inflammation in muscle-related inflammatory conditions, rheumatoid arthritis, osteoarthritis and ankylosing spondylitis [1,2]. Aceclofenac acts by inhibiting cyclooxygenase during prostaglandin synthesis and thereby exhibiting its antiinflammatory effect [3,4].

Storage of drugs may cause physico-chemical degradation. Stability testing of an active pharmaceutical ingredient is therefore performed under various conditions and is required to document these results in drug development process. Identification, characterization of degradation products is required according to guidelines issued by various regulatory authorities and International Conference on Harmonization (ICH) and other regulatory authorities [5-7]. Even small amounts of impurities may affect the quality of pharmaceutical products and could produce unwanted evil effects on the health of patients taking such drugs. So methods indicating the stability are important to assess the impurities formed during the storage [8].

To the best of our knowledge, there is only one report on the stress stability study of aceclofenac in tablet dosage form by HPLC. The study showed that the degradant formed is diclofenac by comparison of its standard chromatogram in both acidic and basic stress conditions [9]. Other reports [10-15] were found on simultaneous estimation of aceclofenac along with other NSAIDs by HPLC . All the above cited literature revealed that the drug is unstable under various stress conditions tested and few other degradants were formed. However, they were not characterized. This study was performed to know about the degradation pattern and to characterize the degradants by isolating each of them using various spectroscopic techniques.

EXPERIMENTAL

Aceclofenac was obtained as a kind gift sample from an industry (Hyderabad, India). HPLC grade solvents and buffers were used. Water used was Milli-Q grade.

All the analysis was performed on an Agilent 1290 Infinity LC System (Agilent, Santa Clara, CA,USA) with ACQUITY BEH C18 (2.1 × 50 mm, 1.7 µm particle size) column; column

temperature at 45 ºC; Binary pump with mobile phase A: 0.1 % formic acid in water; mobile phase B: 100 % acetonitrile; T/% of B: 0.0/2.0, 0.5/2.0, 3/30, 8/60, 11/98, 13/98, 14/2, 15/2; Diluent: mobile phase; flow rate 0.6 mL/min; Detection: 215 nm.

Preparative HPLC equipped with Waters Quaternary gradient module 2545, Waters Photo Diode Array detector module 2998; Waters Auto sampler 2707; Waters Fraction collector III (Waters Corp., Milford, MA, USA); column: Kromasil C-18 (150 × 25 mm) 10µ, mobile phase A: 0.1 % formic acid (aq.); mobile phase B:acetonitrile: T % of B: 0.0/30, 8.0/70, 11/70, 11.1/98, 13/98, 13.1/30, 15/30; flow rate : 20 mL/min.

Samples were analyzed with Waters micro mass Q-TOF equipped with an electrospray ionization (ESI) source. Samples were operated in positive and negative mode to enable to detection of degradants. Leucine encephalin (*m/z*: 555.62268 Da) was used as reference lock mass calibrant to achieve the typical mass accuracies with Mass Lynx software. The optimal conditions of analysis were as follows: capillary voltage of 3.5 kV, sample cone at 25 V and the scan range from 100 to 2200 Daltons. The source temperature was 120 ºC and the desolvation temperature set at 350 ºC. Nitrogen was used as nebulization gas at flow rate of 750 L/ h.

NMR spectra of the degradation impurities were recorded in DMSO-d_6 (Cambride Isotope Limited) on 400 MHz Bruker Avance-III HD NMR spectrometer equipped with broad band observe (BBO) probe. The ^{1}H and ^{13}C chemical shifts are reported on δ scale in ppm, relative to tetramethyl silane (TMS) as internal standard. The spectra were set to δ 0.00 ppm in ^{1}H NMR (TMS) and δ 39.50 ppm in ^{13}C NMR (DMSO-d_6).

Stress methods: The drug was subjected to stress as per ICH guideline Q1A (R2) [5] to identify the potential degradants those could be generated during the storage. The details of the stress methods are shown in Table-1.

TABLE-1
STRESS CONDITIONS FOR OPTIMUM DEGRADATION

Stress condition	Concentration of stressor	Exposure condition	Duration
Hydrolysis	Acid-0.5 N HCl	Reflux-50 °C	12 h
	Base-0.5 N NaOH	Reflux-50 °C	12 h
Oxidation	5 % H_2O_2	Reflux-50 °C	12 h

RESULTS AND DISCUSSION

After 12 h of reflux, degradations were observed. 1 mL of the resultant acid, base and peroxide mediated degradation solution were dissolved in mobile phase and a sample of 10 µL was subjected to LC-MS analysis. Drug solution treated with acid showed one degradant and base showed three degradants (Fig. 1). In that one degradant product (DP-2) is same for both acid and base degradation study. No degradation of the drug was observed in peroxide treated drug solution. Acid and base treated solutions were taken up for isolation of all the three detected degradants.

Isolation of acid and base degradation products: As describes above the methods used in preparative HPLC for isolation of degradants. The fractions corresponding to the peaks in each study were collected and dried by lyophilization. The

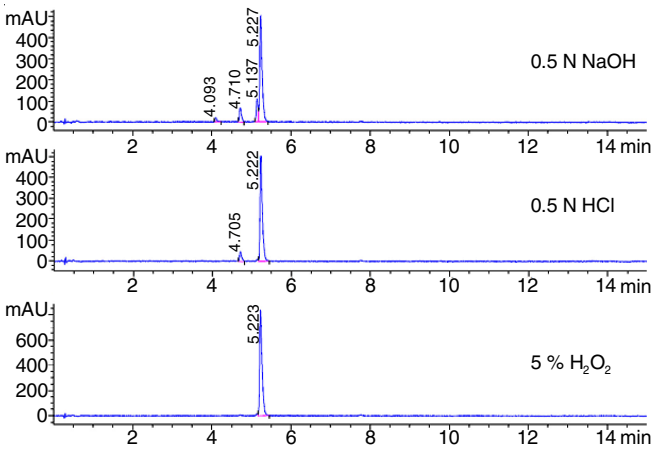

Fig. 1. Acid, base and hydrogen peroxide degradation chromatograms of aceclophenac drug substance

products were labeled as **DP-1**, **DP-2** and **DP-3**. The extensive analysis of HRMS and 1D, 2D NMR data confirmed the structures of the degradation products. Literature survey revealed that **DP-1**, **DP-2** and **DP-3** were already reported. However, this is the first report of **DP-1** as a degradation product of aceclofenac. Structures of aceclofenac and its degradation products in various stress conditions are shown in Fig. 2.

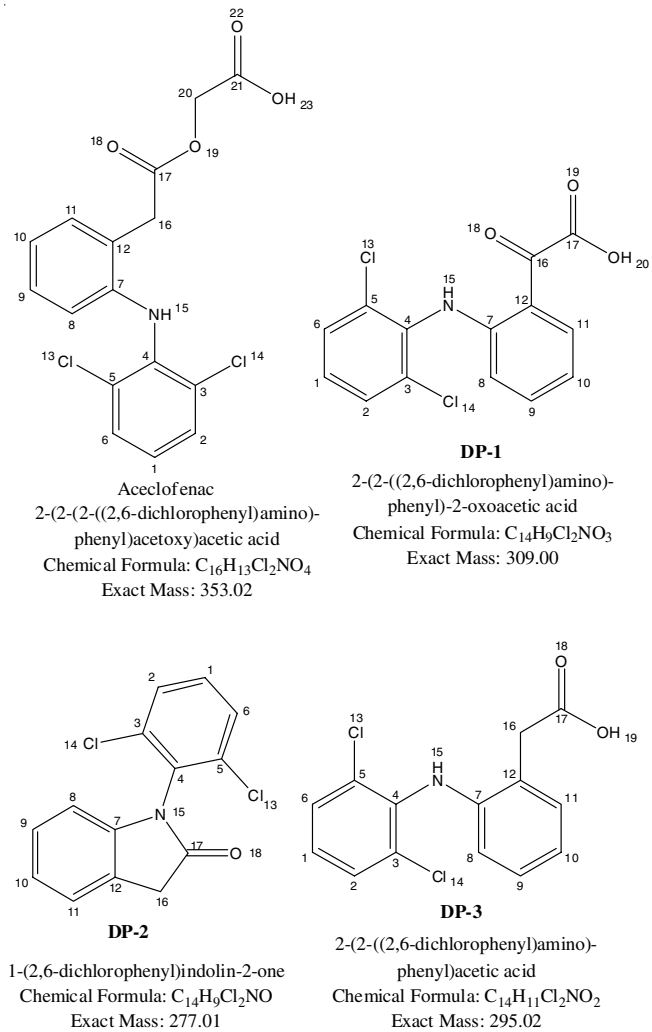

Fig. 2. Structures of aceclofenac drug substance and its degradation products

Structure elucidation of degradation product (DP-1): The HRMS showed a deprotonated molecular ion peak at *m/z* 307.9887 $[M-H]^-$ corresponding to m.f. $C_{14}H_9NO_3Cl_2$. The ^1H NMR spectrum revealed that the DP-1 had seven aromatic protons and one NH exchangeable proton. Carboxylic acid proton was not observed as degradant having moisture. H-16 and H-20 protons (Fig. 2) of aceclofenac drug substance was not observed in DP-1. The ^{13}C NMR spectrum showed 14 aromatic carbons. One of carbon observed at 192.6 ppm indicating that DP-1 having keto-carbonyl group which was not present in drug (aceclofenac). The HSQC analysis revealed that there are 7 methines in the compound. In HMBC experiment, H-11 (7.65 ppm) correlated with 16th position carbonyl group at 192.6 ppm (Fig. 3) indicating that 16th position CH_2 group of drug (aceclofenac) was converted to carbonyl group. All these analysis supporting to structure of DP-1 as shown in Fig. 2. ^1H and ^{13}C chemical shift values was assigned as shown in Table-2 by using ^1H, ^{13}C, COSY, edited HSQC and HMBC experiments.

Structure elucidation of degradation product (DP-2): The HRMS showed a protonated molecular ion peak at *m/z* 278.0140 $[M+H]^+$ corresponding to m.f. $C_{14}H_9NOCl_2$. The ^1H NMR spectrum revealed that DP-2 had 7 aromatic protons and one aliphatic CH_2 protons. DP-2 degradant don't have exchangeable protons of NH and acid protons of drug substance (aceclofenac). The ^{13}C NMR spectrum showed 13 aromatic carbons and one aliphatic carbon. One of carbon observed at 173.2 ppm belong to amide carbonyl group HSQC analysis revealed that there are 7 methines and one methylene in the compound. In HMBC experiment, H-16 (3.89ppm) correlated with C-17 (173.2 ppm), C-7 (142.8 ppm), C-11 (124.9ppm) and C-12 (124.6 ppm) as shown in Fig. 4. All these analysis supporting to structure of DP-2 as shown in Fig. 2. Proton and carbon chemical shift values are assigned as shown in Table-2 by using 1D and 2D NMR data. The compound was already reported earlier as a degradant. However, its spectra data is not published [14].

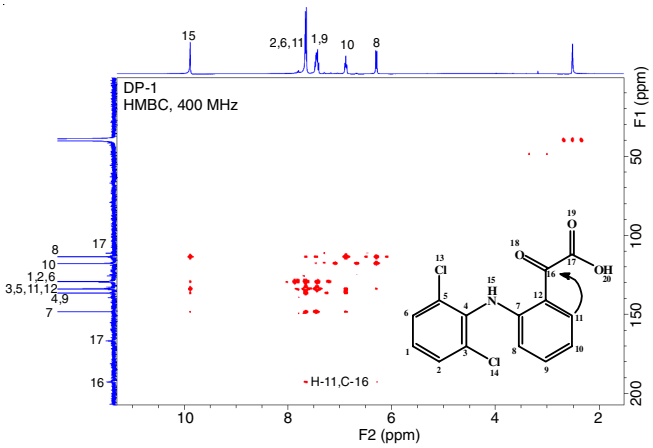

Fig. 3. ^{13}C HMBC spectrum of **DP-1**

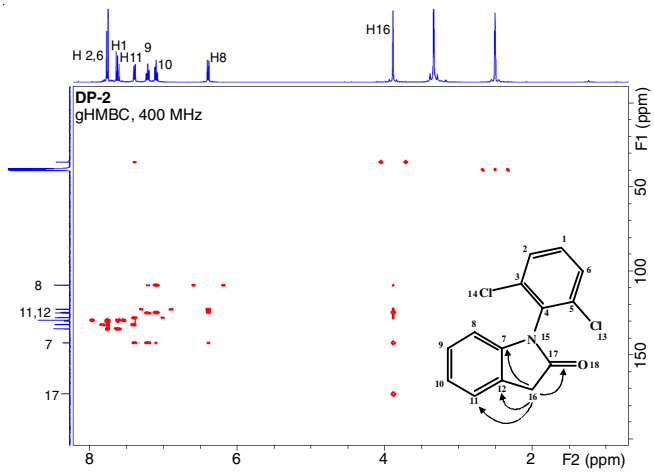

Fig. 4. ^{13}C HMBC spectrum of **DP-2**

Structure elucidation of degradation product (DP-3): The HRMS showed a deprotonated molecular ion peak at *m/z* 294.0088 $[M-H]^-$ corresponding to m.f. $C_{14}H_{11}NO_2Cl_2$. The

TABLE-2
^1H AND ^{13}C NMR DATA OF ACECLOFENAC AND ITS DEGRADATION PRODUCTS

Assignment	Aceclofenac		DP-1		DP-2		DP-3	
	^1H(PPM)	^{13}C (PPM)	^1H(PPM)	^{13}C (PPM)	^1H(PPM)	^{13}C (PPM)	^1H(PPM)	^{13}C (PPM)
1	7.19	125.9	7.43	129.1	7.62	132	7.17	125.5
2,6	7.51	129.1	7.65	129.2	7.76	129.3	7.51	129.1
3,5	–	130.9	–	133.7	–	134.4	–	129.9
4	–	137.2	–	136.4	–	129.9	–	137.1
7	–	142.9	–	148.3	–	142.8	–	142.7
8	6.3	116.2	6.29	113.5	6.39	108.5	6.29	116
9	7.07	127.8	7.46	136.4	7.21	127.8	7.05	127.5
10	6.87	120.8	6.88	117.7	7.09	122.9	6.86	120.8
11	7.26	130.7	7.65	133.8	7.39	124.9	7.21	130.9
12	–	123.1	–	133.74	–	124.6	–	124.1
13,14	–	–	–	–	–	–	–	–
15	6.95	–	9.88	–	–	–	7.3	–
16	3.91	36.8	–	192.6	3.89	35.1	3.7	37.9
17	–	171	–	166.7	–	173.2	–	173.4
18	–	–	–	–	–	–	–	–
19	–	–	–	–	–	–	12	–
20	4.65	61.1	–	–	–	–	–	–
21	–	169	–	–	–	–	–	–
22	–	–	–	–	–	–	–	–
23	13.1	–	–	–	–	–	–	–

^{1}H NMR spectrum revealed that DP-3 had 7 aromatic protons, 1 aliphatic CH_2 group, two exchangeable protons of one NH and one carboxylic acid. All protons of drug substance (aceclofenac) were observed in DP-3 except H-20 proton (Fig. 2). The ^{13}C NMR spectrum showed 13 aromatic carbons and one aliphatic carbon. One of carbon observed at 173.4 ppm indicated that DP-3 having acid carbonyl group. HSQC analysis revealed that there are 7 methines and 1 methylene in the compound. In HMBC experiment, H-16 (3.7 ppm) correlated with 17th position carbonyl group at 173.4 ppm, C-7 (142.7 ppm), C-11 (130.9 ppm) and C-12 (124.1 ppm) as shown in Fig. 5 indicated that 16th position CH_2 group is present in between of benzene group and carboxylic acid. This was found to be diclofenac and has already been reported as a degradant in the stability studies earlier [9]. All these analysis supporting to structure of DP-3 as shown in Fig. 2. ^{1}H and ^{13}C chemical shift values was assigned as show in Table-2 by using ^{1}H, ^{13}C, COSY, edited HSQC and HMBC experiments.

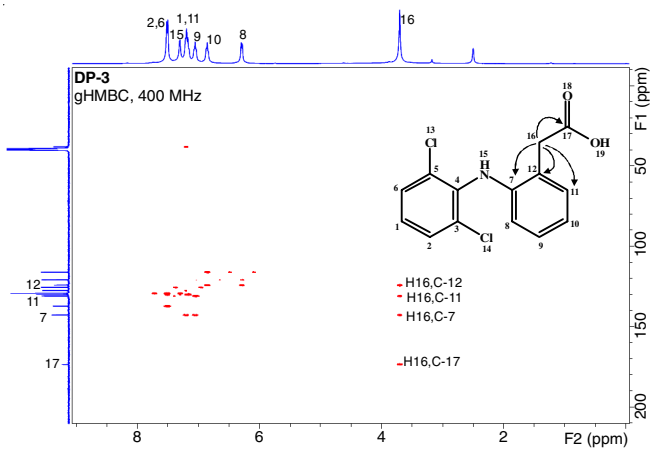

Fig. 5. ^{13}C HMBC spectrum of **DP-3**

Conclusion

Forced degradation of aceclofenac was performed as per the ICH guidelines. The drug was subjected to acid, base and oxidative stressed conditions and three degradation products were found to be formed. All the products were selectively separated and fully characterized by various 2D NMR and mass spectroscopic methods experiments. The degradation products **DP-2** and **DP-3** were reported as the impurities of aceclofenac [9-14]. However, to the best of our knowledge, this is the first report on the structure characterization of degradation product (DP-2).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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